THE HYDROLYSIS OF SUCROSE BY THE GASTRIC JUICE

BY

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Robert McClaughry Hill

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I. Introduction.

The question of the opportunity for and extent of hydrolysis of cane sugar in the stomach normally is still an open one. While it is generally conceded that the acidity of the gastric juice is sufficiently high to permit of a considerable degree of hydrolysis over a prolonged period of time, the actual extent to which this acid hydrolysis may proceed under normal conditions of diet in man has never been the subject of careful experimental study. Such a study has been attempted in the present instance.

Röbner (1) in 1859 and Claude Bernard (2) stated that gastric juice had the power of inverting cane sugar. Experiments carried out by Hoppe-Seyler (3) in 1856 and Külz (4) 1874 indicate that the gastric juice has no such power when the sugar is fed in moderate quantities. Hoppe--Seyler, however, found appreciable inversion when large quantities of sugar were fed. In 1887 Seegan (5) carried out sugar feeding experiments on dogs. The dogs were fed 100 g. of cane sugar daily for from 7 to 8 days and at the end of that period were killed and examinations made of the contents of the stomach and duodenum. Since glucose was found in the stomach and no sucrose was found in the duodenum, Seegan came to the conclusion that sucrose is completely inverted in the stomach. Three years later, Voit (6) carried out very similar experiments with rabbits, to which were administered 30 g. of sucrose. Six and a half

hours later they were killed and examinations made of the different portions of the alimentary tract. Analysis showed that of the sugar remaining in the stomach 90% was hydrolyzed and only 2 mg. of sucrose in the duodenum, an amount thought to be within the experimental error of the methods employed. The conclusion drawn was the same as that of Seegan, that inversion was practically completed in the stomach. Voit was able, however, to recover from the entire alimentary tract only 16% of the sucrose fed. In consideration of this fact and of the short time that carbohydrates are now known to remain in the stomach, it seems that the evidence on which the conclusion is based is insufficient. In 1898 Ferris and Lusk (7) carried out a series of hydrolysis experiments, in vitro, with 1% to 5% of sucrose solutions to see whether the normal gastric concentration of hydrochloric acid (0.2-0.3%) was sufficient to account for the inversion found in Voit's experiments. While the inversion in 6.5 hours was 10% to 20% lower than that found by Voit in rabbits, they concluded that the inversion in the stomach could be completely accounted for by the concentration of free acid. In 1902 Widdicombe (8) reported the presence of an active sucrase in the gastric juice of dogs and pigs, but Lusk (9) in 1904 repeated these experiments and was unable to corroborate his findings.

In regard to the action of gastric juice on sucrose the statements in the current textbooks are very contradictory. Some of the authors recognize the unsettled state of the

question but others make positive statements, - hardly justified by our present knowledge - the contradictory nature of which has given rise to considerable confusion. The following quotations illustrate the widely varying treatment of the subject: "--the hydrolysis of the carbohydrates of the food may be continued, to some extent at least, by the hydrochloric acid of the stomach. This chemical hydrolysis, however, if it occurs, is of less importance than the action of the gastric enzymes upon proteins, milk and fats." (Bainbridge and Menzies (10)). "While proteids are practically the only nutrients digested in the stomach, it is also true that none of the preparatory actions on other nutrients take place here.----- Sugar and certain salts may also be dissolved here by the water and acid of the gastric juice." (Eddy (11)). "Gastric juice has no direct action on carbohydrates." (Huxley (12)). In contrast to these we have --"By the action of the hydrochloric acid (of the gastric juice) certain changes are induced in the food stuffs. Cane sugar is inverted to glucose and fructose." (Starling (13)). "It (gastric juice) inverts cane sugar into glucose and fructose." (Halliburton (14)). According to Mathews (15) "To what extent cane sugar is inverted in the stomach will depend on the length of time it remains there after free acid appears. This time is normally so short that probably little inversion occurs," and Hammersten (16) "The statements in regard to the ability of gastric juice to invert cane sugar are very contradictory. At least

this action of the gastric juice is not constant, and if it is present at all it is probably due to the action of acid."

Those who have held that the hydrochloric acid of the gastric juice was the active agent in the hydrolysis of sucrose in the stomach have based their belief on experiments carried out in vitro with sucrose in acid solutions of the strength assumed to be present in the stomach. Based on this assumption the inversion could not be very great if the short time that sucrose remains in the stomach under normal conditions is considered. Those who have believed that there are other factors concerned have argued that on postmortem examinations after sucrose feeding, sucrose and reducing sugar are found in the stomach whereas only invert sugar is found in the duodenum. This seemed to prove that the sucrose was all hydrolyzed before it passed the pylorus.

All of our knowledge of the extent of inversion of sucrose in the stomach has been obtained from hydrolysis by hydrochloric acid in vitro and from postmortem examinations after sucrose feeding. At best the results of such postmortem examinations are very doubtful indices of the actual physiological processes under normal conditions. For these reasons it was thought that it would be profitable to carry out a series of direct experiments on the inversion of sucrose in the human stomach. The Rehfuss stomach tube makes such an examination especially feasible, not only because the examination is made under normal conditions of digestion, but because it is possible to follow the course of the inversion by making use of the "fractional" method of analysis.

II. Methods.

The Benedict-Osterberg (17) modification of the Benedict blood sugar method was used for the determination of the glucose in these experiments. The picric acid employed was purified according to the procedure of Folin and Doisy (18). Picramic acid purified by the method of Egerer (19) was used as the color standard. The sucrose used in these experiments was purified from the best commercial granulated sugar. The method of purification was the following: A thick syrup was made by dissolving the sugar in water which, to avoid any hydrolysis, was not heated. The syrup was then poured slowly into 95% alcohol from which sucrose precipitated out in very fine crystals. A second precipitation was made and the sugar then dried with suction on a Buchner funnel and finally in a vacuum desiccator. The sucrose purified in this way which we used in our experiments when tested in the polarimeter showed a purity of 99.98%.

The possibility was suggested that the picrate-picric acid reagent might invert some of the sucrose and thus cause high results. To test this, known amounts of sucrose were added to a standard solution of glucose containing one mg. of glucose in 4.0 cc. The glucose was then determined in the solutions made up in the same way. The results given in Table I show that the glucose content is not altered by the presence of sucrose and that the picrate-picric acid reagent does not invert any of the sucrose present.

III. The Action of Hydrochloric Acid on Sucrose in Vitro.

In order to determine to what extent hydrochloric acid in the concentrations found in gastric juice would invert sucrose, hydrolysis experiments in vitro were run. Ferris and Lusk (7) have carried out such determinations. We repeated these experiments using essentially the same procedure, the details of which are indicated in Table II. Erlenmeyer flasks containing 20.0 cc. of 0.5% and 1.0% sucrose solution with the desired hydrochloric acid concentration were incubated for two hours at 37° C. and samples withdrawn at stated intervals for analysis. The samples were immediately neutralized exactly to methyl red-methyl blue, made up to definite volume, and the glucose determined in aliquots. The results of these analyses are shown in Table II. Our figures agree in general with the results of similar experiments of Ferris and Lusk (7), following, as they point out, Wilhelmy's law of chemical change. As the action of the acid continues, the concentration of the sucrose decreases so that the rate of the reaction becomes less as it proceeds. In the case of the 1.0% sucrose in 0.7% hydrochloric acid if the rate of the reaction remained the same the inversion would be 8.4% at the end of one hour and 16.8% at the end of two. Actually the inversion at the end of these periods is 8.2% and 15.6% showing the progressive decrease in the rate of inversion.

IV. The Action of Gastric Juice on Sucrose in Vivo.

The general procedure used by Okey (20) in her investigation of the hydrolysis of inulin in the stomach was followed in these experiments. The subjects were healthy men and women, students in the laboratory. The experiments were carried out on the fasting subject, no food having been consumed since the evening meal of the previous day. In the earlier experiments a "meal" was given consisting of the whites of three eggs cooked "soft". As an experiment (Table III) carried out with the egg white meal without sucrose showed that the egg white contained an appreciable amount of glucose, for which corrections had to be made, in the later experiments 50 g. of cottage cheese were used as the test meal. The cottage cheese was repeatedly triturated with water (7-10 times) and drained through cheesecloth until 5 cc. of the wash water gave no appreciable reduction with 10 cc. of Fehling's Solution. About fifteen minutes after the meal was eaten the stomach tube was taken, and after five minutes 5.0 g. of sucrose in 150 cc. of distilled water were given through the tube and the tube rinsed with 50 cc. of distilled water. In the later experiments 100cc. of the distilled water were given before the sucrose and after five minutes a sample withdrawn to test for reducing substances. After the introduction of the sugar, three samples were removed at twenty minute intervals for examination. The "free" and "total" acid were titrated against standard sodium hydroxide using dimethylaminoazobenzene and phenolphthalein as indicators.

An aliquot was neutralized immediately and the protein precipitated by adding the picrate-picric acid reagent with which it was made to 25 cc. The glucose was then determined by the Benedict-Osterberg modification of the Benedict method. These results represent the amount of reducing sugar present due to the normal action of the gastric juice. A second aliquot was incubated at 37° C. for two hours and the reducing sugar determined in the same way. The results in this case showed the amount of inversion possible if the normal gastric juice were afforded the opportunity for prolonged action on the sucrose. To a third portion hydrochloric acid was added to give a final concentration of 1.0% hydrochloric acid and the mixture was hydrolyzed by boiling for one hour. The boiling in acid solution should completely invert the sucrose and the subsequent determination of the reducing sugar would then show the total carbohydrate, sucrose plus invert sugar, present. Since regurgitation from the intestine, at intervals, is now known to accompany gastric digestion normally, it was thought that the sucrase of the intestinal juice might have an appreciable effect in inverting sucrose in the stomach. To test this a sample was withdrawn from the stomach before the sucrose was introduced, and neutralized. Sucrose was added and the mixture incubated for two hours. Any hydrolysis in this test would indicate the presence of sucrase since other hydrolyzing factors were eliminated. In none of these was there any hydrolysis, showing the absence of active sucrase.

Five experiments were carried out with the egg-white meal and three with the cottage cheese. In all seven subjects were studied. Early in the experiments where the egg-white meal was ingested (Table IV) there is apparently a considerable hydrolysis which the free acid present cannot completely account for. However, when these figures are corrected for the glucose content of the egg-white (Table III) the hydrolysis represented by the corrected figure can be easily accounted for by the free acid concentration. This corrected figure also correspond very closely to that obtained with the cottage cheese meal which contained only a negligable amount of glucose. After two hours incubation there is obtained a hydrolysis closely parallel with the hydrolysis due to hydrochloric acid alone in similar concentrations as shown in the experiments recorded in Table II. The total sugar present, as shown by the completely hydrolyzed samples, indicates that the sugar has almost completely left the stomach at the end of one hour. Even though the per cent of hydrolysis is comparatively large, it is in reality of little consequence because of the small quantity of sugar remaining in the stomach at that time.

These experiments show a slight hydrolysis of the sucrose in the sample taken from the stomach twenty minutes after ingestion and a small increase in the hydrolysis in the samples taken later. The extent of hydrolysis in the stomach can be of no practical importance because of the short time the sugar remains there and in every case it can be almost completely accounted for by the action of the free acid present.

V. The Action of Gastric Juice on Sucrose in Vitro.

To confirm our findings in the feeding experiments a series of tests were carried out on the action of gastric juice on sucrose in vitro. The egg-white meal was fed in all of these and only one sample of gastric juice was withdrawn. This was removed 40 minutes after the meal which is the average time of maximum acidity under the conditions of these experiments. In one portion the acidity of the gastric juice was determined, in a second reducing sugar was analyzed for. The latter figure shows the amount of glucose in the gastric contents and was used as a correction to be applied in the following inversion tests. To each of four 10 cc. aliquots 50 mg. of sucrose were added. The first portion was incubated two hours. This is comparable with the similar tests in the feeding experiments, and gave similar results as may be seen by comparing the figures obtained with the incubated samples in Tables IV. and V. Since the attempt, described in Section IV., to demonstrate the activity of regurgitated intestinal sucrase in the stomach did not give positive results, a further test was made. For this purpose the second portion of gastric juice was heated to boiling before any sucrose was added. On cooling, 50 mg. of sucrose were added and it was then incubated for two hours. The heating would kill any enzyme that might be present and the inversion should be due solely to the free acid concentration. The third aliquot was not heated but was neutralized before the addition of the sucrose and then incubated. If any inversion

occurred in this case it would necessarily be due to factors other than the acidity and would indicate the presence of enzymes. The fourth aliquot was both boiled and neutralized before the sucrose was added. This was run as a control and no inversion would be expected.

Inspection of Table VI. shows that the results of this series of experiments bear out the findings in the feeding tests. Boiling the gastric juice without neutralization has no effect on its power to invert sucrose while neutralization alone completely takes away that power. This shows that no enzyme action is concerned in the inversion and that the presence of free acid is necessary for it to take place. Comparison with the results recorded in Table II. show that the inversion is practically identical with that in aqueous solutions of the same hydrochloric acid concentration, and therefore that the free acid concentration of the gastric juice is sufficient to explain all of the hydrolysis.of sucrose that takes place in the stomach. These experiments further show that if any sucrase is carried into the stomach by regurgitation from the intestine it does not remain active long enough to have any effect in the gastric hydrolysis of sucrose.

VI. The Retention of Glucose and Sucrose in the Stomach.

During the course of our investigation the question arose as to whether glucose and sucrose leave the stomach at the same rate. If glucose leaves more rapidly than sucrose it is evident that analysis of the gastric contents alone will give figures that are too low for the per cent of inversion. Five grams of glucose were given under the same conditions as in the sucrose experiments and samples removed at twenty minute intervals were neutralized immediately to methyl red--methylene blue. The glucose was then determined as in the previous experiments. The results of this test are shown in Table VII. In Table VIII the average speed of evacuation of sucrose from the stomach in all the sucrose feeding experiments is compared with the speed of evacuation of glucose. As a basis of comparison we have taken the analysis of 5 cc. aliquots of the first sample, withdrawn twenty minutes after the meal was eaten. The figures in the table show the percentage of sugar still present in the stomach, after forty and sixty minutes, computed on this basis. The table also shows the free acidity of the glucose feeding experiment compared with the average free acidities of the sucrose experiments. The glucose appears to be leaving the stomach more rapidly in the first period but the free acidity in the case of the glucose is correspondingly higher and this would lead us to expect a more rapid evacuation. It is recognized that in these experiments very close agreement cannot be expected because of the varying extent of dil-

ution of the sugar solutions by the gastric juice, and further since only one subject was studied the tendency to individual variation must be considered. However, we believe that this shows clearly that there is no material difference in the rate at which glucose and sucrose leave the stomach under the conditions of our experiment.

VII. Summary.

(1) Hydrochloric acid of the strength normally found in the stomach has the power of inverting sucrose in appreciable quantities in from one to two hours.

(2) The inversion of sucrose in the human stomach may be explained solely by the action of the hydrochloric acid present. This inversion is too small to be of any consequence in the short time that the sucrose remains in the stomach.

(3) The inverting action of human gastric juice in vitro is essentially the same as that of hydrochloric acid solutions of the same concentration.

(4) We were unable to demonstrate the presence of any sucrase of either gastric or intestinal origin in gastric juice.

(5) Sucrose and glucose leave the stomach at essentially the same rate.

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Table I.

The Determination of Glucose in the Presence of Sucrose.

Glucose in 4cc.	Sucrose Added	Glucose in 4cc. Found
mg.	mg.	mg.
1	0	1.00
1	5	1.01
1	10	0.99
1	15	0.99
1	20	0.99
1	25	0.99

Table II.

Acid Hydrolysis of Sucrose.

Sucrose	Hydrochloric Acid	Reducing Sugar Found.		
		.5 hour	1 hour	2 hours.
%	%	mg.	mg.	mg.
0.5	0.1		3.7 (7.4)	5.4 (10.8)*
0.5	0.2		5.0 (10.0)	7.5 (15.0)
0.5	0.26		6.0 (12.0)	10.9 (21.6)
1.0	0.1		2.8 (5.6)	5.5 (11.0)
1.0	0.1	1.8 (3.6)	3.0 (6.0)	5.6 (11.2)
1.0	0.2	2.1 (4.2)	4.1 (8.2)	7.8 (15.6)
1.0	0.4	5.8 (11.6)	9.7 (19.4)	19.0 (38.0)

*The figures in parentheses show the per cent of hydrolysis.

Table III.

Glucose in the Gastric Contents After Feeding the Egg-White Meal Without Added Sucrose*

Time After Ingestion.	Free Acidity	Total Acidity	Reducing Sugar per 5 cc. Gastric Contents.	
min.	cc. N/10 acid	cc. N/10 acid	Before Incubation	After Incubation.
20	10.9	27.6	2.5	2.4
40	50.8	70.8	1.7	1.9
60	54.4	77.6	1.9	2.1

* Subject C. B.

Table IV.

Egg-White Meal.

Subject	Time After Ingestion	Free Acidity	Total Acidity	Reducing Sugar per 5cc. Gastric Contents	Reducing Sugar per 5cc. After Incubation	Total Sugar per 5cc. After Acid Hydrolysis	Inversion In Stomach	Inversion After Incubation.
	min.	cc.N/10 acid	cc.N/10 acid	mg.	mg.	mg.	%	%
J. J.	20	1.4	29.3	3.5	4.3	34.5	10.2	12.3
	40	18.6	24.3	1.8	2.0	11.1	16.2	18.0
	60	18.0	28.6	1.1	1.5	6.0	18.6	25.2
H.	20	14.1	31.4	2.2	4.0	26.8	8.3	14.9
	40	21.4	34.3	1.7	2.0	9.3	18.1	21.5
	60	31.4	42.0	1.1	1.4	7.7	14.3	18.2
M.	20	2.8	47.9	4.0	2.7	36.5	11.0	7.4
	40	17.9	54.3	3.8	3.1	32.5	11.5	9.5
	60	34.6	60.3	3.1	3.2	19.3	16.0	16.6

Continued

Table IV.-Continued.

Egg-White Meal.

Subject	Time After Ingestion	Free Acidity	Total Acidity	Reducing Sugar per 5cc. Gastric Contents	Reducing Sugar per 5cc. After Incubation.	Total Sugar per 5cc. After Acid Hydrolysis	Inversion In Stomach	Inversion After Incubation.
	min.	cc. N/10 acid	cc.N/10 acid	mg.	mg.	mg.	%	%
C. B.	20	25.0	49.0	6.0	7.1	53.5	11.2	13.3
	40	34.0	84.0	5.7	5.9	36.5	15.6	16.2
	60	36.0	75.0	4.1	6.1	20.5	19.8	29.8
W. P. B.	20	25.0	33.0	3.1	3.9	49.5	6.3	8.0
	40	42.0	60.0	2.0	3.2	17.3	11.6	18.5
	60	41.0	56.0	---	0.8	4.0	----	19.0
				1.9*				

*Sample taken before sucrose administration.

Table V.

Cottage Cheese

Subject	Time after Ingestion	Free Acidity	Total Acidity	Reducing Sugar per 5cc. Gastric Contents.	Reducing Sugar per 5cc. After Incubation.	Total Sugar per 5cc. After Acid Hydrolysis	Inversion In Stomach	Inversion After Incubation
	mins.	cc. N/10 acid	cc.N/10 acid	mg.	mg.	mg.	%	%
C. B.	20	34.3	42.9	1.7	8.0	57.5	3.0	13.9
	40	44.3	57.2	4.0	12.3	63.0	6.3	19.5
	60	37.2	45.8	---	2.9	17.9	---	16.2
J. S.	20	26.5	37.9	2.2	10.4	100.0	2.2	10.4
J. B. H.	20	17.2	20.6	0.6	2.1	31.8	1.9	6.6
	40	20.6	27.2	1.5	1.7	14.0	8.2	12.1
	60	25.7	----	0.8	1.0	3.6	20.8	28.0

Table VI.

The Hydrolysis of Sucrose by Gastric Juice in Vitro.

Subject	Gastric Juice	Sucrose Added	Period of Incubation.	Free Acidity	Total Acidity	Reducing Sugar per 5 cc. Gastric Juice.	Notes.
	cc.	mg.	hours	cc.N/10 acid	cc.N/10 acid	mg.	
M. L. B.	10	50	2	22	34	2.6	
	10	50	2	22	34	2.6	Sample boiled before addition of sucrose
	10	50	2	22	34	1.1	Neutralized and boiled before addition of sucrose
W.P.B.	10	50	2	20	35	4.0	
	10	50	2	20	35	3.9	Boiled before addition of sucrose
	10	50	2	20	35	3.8	Neutralized before addition of sucrose
	10	50	2	20	35	3.7	Boiled and neutralized before addition of sucrose

continued

Table VI. -- Continued.

Subject	Gastric Juice	Sucrose Added	Period of Incubation.	Free Acidity	Total Acidity	Reducing Sugar per 5 cc. Gastric Juice	Notes
	cc.	mg.	hours	cc.N/10 acid	cc.N/10 acid	mg.	
F. H. T.	10	50	2	44	68	7.5	
	10	50	2	44	68	7.5	Boiled before addition of sucrose
	10	50	2	44	68	3.0	Neutralized before addition of sucrose
	10	50	2	44	68	4.3	Boiled and neutralized before addition of sucrose
	10	0	0	44	68	4.1	Without added sucrose

The egg-white meal was used.

The last test with each subject gives the correction to be applied for the glucose content of the meal.

Table VII.

The Retention of Glucose in the Stomach.

Subject	Time After Ingestion	Free Acidity	Total Acidity	Glucose in 5cc. Stomach Contents.
S. S. G.	min.	cc.N/10 acid	cc. N/10 acid	mg.
	20	34.7	43.9	68.1
	40	57.2	77.1	31.3
	60	50.0	----	18.8

Table VIII.

The Rate of Evacuation of the Stomach.

Comparison of Glucose and Sucrose.

Time After Ingestion.	Free Acidity cc.N/10 acid		*Per cent Remaining in Stomach	
	Glucose	Sucrose	Glucose	Sucrose
20	----	----	100	100
40	57.2	28.3	46	56
60	50.0	32.0	28	26

* The amount of carbohydrate present at the end of twenty minutes is taken as a basis for the calculation of the per cent of sugar remaining in the stomach in the later periods.